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Control of Cnida Discharge: III. Spirocysts are Regulated by Three Classes of Chemoreceptors

GLYNE U. THORINGTON AND DAVID A. HESSINGER[1]

Department of Physiology and Pharmacology, School of Medicine, Loma Linda University, Loma Linda, California 92350

Abstract. Spirocysts are two to three times more abundant than nematocysts in the feeding tentacles of acontiate sea anemones. Despite their prevalence, little experimental work has been done on the discharge of spirocysts because of the difficulty in detecting and counting them after they have discharged. To circumvent this problem, we have developed a simple, reliable, enzyme-linked lectin sorbent assay (ELLSA) for quantifying discharged spirocysts. With this method, we have shown that the discharge of spirocysts, like that of mastigophore nematocysts, is chemosensitized in a dose-dependent manner by three classes of low molecular weight substances, typified by N-acetylneuraminic acid (NANA), glycine, and certain heterocyclic amino compounds, such as proline and histamine. We also show that spirocysts exhibit considerable agonist-specific variation in the dose-responses of discharge, suggesting the existence of multiple populations of spirocyst-bearing cnidocyte/supporting cell complexes (CSCCs). Our findings call into question commonly held views regarding the respective roles of spirocysts and mastigophore nematocysts in the retention of captured prey.

Introduction

The cnidom of the feeding tentacles of acontiate sea anemones, including *Aiptasia pallida*, consists of three types of cnidae: spirocysts; microbasic p-mastigophore nematocysts, and basitrichous isorhiza nematocysts (Hand, 1955) in approximate ratios of 3:1:0.3, respectively (Bigger, 1982; Watson and Mariscal, 1983). Cnidae function primarily in the capture of prey (Ewer, 1947), in aggression (Purcell, 1977; Bigger, 1982), in defense (Francis, 1973), and in the attachment to appropriate substrates (Mariscal, 1972).

Spirocysts are adherent cnidae found only in zoantharian anthozoans (Mariscal *et al.*, 1978; Mariscal, 1984). An undischarged spirocyst consists of a single-layered capsule containing a long, spirally coiled, inverted tubule of uniform diameter (Mariscal, 1974). The tubule lacks spines, but bears hollow rods that dissociate upon discharge to form a web of fine, adhesive microfibrillae (Mariscal *et al.*, 1977).

Unlike nematocysts, discharged spirocysts are difficult to see under the light microscope due to their non-refractile, transparent capsules (Weill, 1934). Because the tubules of discharged spirocysts entangle extensively (Stephenson, 1929; Skaer and Picken, 1965; Picken and Skaer, 1966; Mariscal, 1974; Mariscal *et al.*, 1977), it is difficult to visually distinguish individual tubules. Thus, it is tedious and time-consuming to visually count spirocysts discharged onto test probes.

To circumvent this difficulty, we developed a simple, sensitive, and reproducible assay to quantify spirocysts discharged onto test probes. The method is based on the recent discovery that the everted tubules of spirocysts have a high affinity for free and conjugated N-acetylated sugars such as occur on mucins, asialomucins, and mucopolysaccharides (Watson and Hessinger, in prep.). The terminal sugars of the unbranched oligosaccharide chains of bovine submaxillary asialomucin are N-acetylgalactosamine. This saccharide binds specifically to the lectin from *Vicia villosa*. Subsequent to binding asialomucin to discharged spirocysts, we determine the number of discharged spirocysts adhering to gelatin-coated test probes by measuring the amount of asialomucin bound to probes using a peroxidase conjugate to the *Vicia* lectin.

We describe a relatively rapid enzyme-linked, lectin

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[1] To whom all correspondence should be addressed.

sorbent assay (ELLSA) to determine the number of spirocysts discharged onto test probes. Using the ELLSA, we show that three classes of agonists sensitize spirocytes to discharge their spirocysts in response to triggering mechanical stimuli. The dose-response curves of spirocyst discharge to the agonists indicate that multiple populations of discharging spirocysts exist, each characterized by different sensitivities to the agonists.

Materials and Methods

Sea anemone maintenance

Monoclonal sea anemones (*Aiptasia pallida*, Carolina strain) were fed and maintained individually in glass finger bowls containing natural seawater at 24 ± 1°C as previously described (Thorington and Hessinger, 1988a).

Experimental animals and test solutions

Prior to each experiment, animals of the same size were starved for 72 h and kept under defined conditions and lighting (Thorington and Hessinger, 1988a). Test solutions of chemosensitizing agonists (N-acetylneuraminic acid, glycine, proline and histamine; Sigma, St. Louis, Missouri) were prepared in natural, filtered (Type 1, Whatman) seawater adjusted to pH 7.6 with 1 *N* HCl or NaOH. Animals were permitted to adapt to changes of medium for 10 min before cnidocyte responsiveness was measured.

Assays of cnidocyte responsiveness

Three methods were used to measure the discharge of cnidae: (1) cnida-mediated adhesive force; (2) microscopic enumeration of discharged microbasic p-mastigophores and spirocysts; and (3) an indirect, solid-state enzyme-linked lectin sorbent assay (ELLSA) of discharged spirocysts.

Cnida-mediated adhesive force. Cnida-mediated adhesive force was measured as previously described (Thorington and Hessinger, 1988a). In principle, this technique involves using a small, gelatin-coated nylon bead attached to a strain gauge via a stainless steal wire shaft. The gel-coated bead is made to contact the tip of a tentacle on an anemone in a finger bowl containing a solution of chemosensitizing agent in seawater. The discharge of cnidae initiated by contact of the probe with the tentacle results in the tubules of the everting cnidae either adhering to or penetrating the gelatin surface. Withdrawing the probe from the tentacle causes the discharged cnidae to exert an opposite and downward force on the probe, which is measured from a gravimetrically calibrated force-transducer connected to a strip-chart recorder. The adhesive force, measured in hybrid units of mg-force (mgf), is the force required to break the cnida-mediated attachment between the probe and the tentacle. It is an aggregate measure of several contributions, including the different kinds of discharged cnidae and the inherent "stickiness" of the tentacle surface, and is proportional to the total number of cnidae discharged onto the probe (Geibel *et al.*, 1988).

Enumeration of discharged mastigophores and spirocysts. Following the measurement of adhesive force, the same gel-coated probes were used to visually count the number of adhering mastigophore nematocysts by methods previously described (Geibel *et al.*, 1988).

Discharged spirocysts were visually counted by the same procedures used for discharge mastigophores. Even with phase contrast optics, however, fully discharged spirocysts were extremely difficult to see and time-consuming to count. To expedite counting of discharged spirocysts adhering to test probes, we developed a fast and reliable micro-assay termed an ELLSA.

Indirect, solid-state enzyme-linked lectin sorbant assay (ELLSA)

This assay for quantifying discharged spirocysts is based upon the observation that the everted tubules of discharged spirocysts bind conjugated N-acetylated sugars with high affinity (Watson and Hessinger, in prep.). In brief, the assay involves first dipping the gel-coated tips of spirocyst-bearing probes into a solution of asialomucin, then into a solution of *Vicia villosa* lectin/peroxidase conjugate, followed by colorimetric measurement of bound peroxidase activity. Some of the N-acetylgalactosaminyl residues on the asialomucin molecule bind to the adhesive "glue" of the everted tubules while the remaining terminal sugars bind the lectin/peroxidase.

Buffers. The following buffers were prepared: Buffer A (0.69 *M* NaCl and 0.25 *M* phosphate, pH 7.6); Buffer B (0.15 *M* NaCl and 0.01 *M* phosphate, pH 6.0 containing 0.02% Tween 20); Buffer C (0.15 *M* NaCl and 0.01 *M* phosphate, pH 6.0); and Buffer D (0.5 M sodium citrate-HCl pH 5.3).

Asialomucin solution. Asialomucin (12 μg/ml; A-0789, Sigma) in filtered seawater was divided into 10 ml aliquots and stored frozen. For assays, a solution of asialomucin (10.8 μg/ml) was prepared by adding nine parts of the stock solution to one part of Buffer D.

Lectin/enzyme conjugate. Horseradish peroxidase conjugated to *Vicia villosa* lectin (E-Y Laboratories, San Mateo, California) was diluted to a final concentration of 1.5 μg/ml in Buffer A. Aliquots of lectin/enzyme conjugate were protected from light and frozen (−20°C) until used. Mannose (50 m*M*) was added immediately prior to using the lectin conjugate to minimize nonspecific interactions between the lectin and the gelatin on test probes.

Enzyme substrate. Hydrogen peroxide (30%; Sigma)

was daily diluted to 3% (v/v) with distilled water and then to 0.3% with Buffer C. The final substrate solution was prepared immediately before use by adding 6 ml of 0.3% H_2O_2 to 0.05 ml of 1% *o*-dianisidine (Sigma) in methanol.

Assay procedure. The wells of flat-bottomed, 96-well microtiter plates (Dynatech) were each rinsed with 200 μl of Buffer B, emptied, and then air dried for 30 min. Test probes were secured to a plastic holder that permitted individual probes to be immersed in the contents of separate wells without coming into contact with the sides or bottom of the wells. All incubations were performed at room temperature. Probes were incubated in the asialomucin solution for 30 min, then rinsed by immersing in individual wells containing Buffer C for 2 min, and finally air-dried for 5 min.

Mucin-treated probes were incubated in separate wells containing 200 μl of lectin/enzyme conjugate for 60 min in the dark. Following a 2-min rinse in Buffer C, they were transferred to wells containing 200 μl of enzyme substrate where they were incubated for 60 min. Following the incubation, the probes were removed and 50 μl 40% sodium azide in Buffer C was added to each of the wells to stop peroxidase activity.

The absorbance of each well was measured at 492 nm using a microtiter well spectrophotometer (Model EL 308, Biotek Instruments, Cambridge, Massachusetts). The mean values of controls, consisting of gel-coated probes, which had not been touched to sea anemone tentacles, were subtracted from the values of individual experimental probes. Probes sputter-coated with gold for 4 min at 15 μA using a Polaron E 5100 sputter coater and then dipped into asialomucin (10.8 μg/ml) in Buffer D were used as external standards to assess reactivity of reagents and to normalize data from test probes to the standard curve, when necessary. The "gold" standard gave absorbances of 0.07 (±0.003 S.E.M.) O.D. at 492 nm. For experimental probes, the absorbance at 492 nm is linearly and directly proportional to the number of discharged spirocysts. Measurements of absorbance are directly converted to the number of discharged adherent spirocysts on a probe by extrapolation from the standard curve.

Results

Optimal dilution of ELLSA reagents

Checkerboard titrations of asialomucin and of lectin peroxidase were performed in microtiter plate wells. The dilutions ranged from 1:3 to 1:81 for asialomucin and 1:8 to 1:5832 for lectin peroxidase. Test probes were gold-coated insect pins (See Materials and Methods) with heads of 0.8 mm diameter. Negative controls were probes treated with 0.01 *M* phosphate buffered saline, pH 6.0. The dilutions chosen were those giving the greatest difference between controls and experimentals. The optimal dilution for asialomucin was 1:27, which is equivalent to 10.8 μg/ml; and for the lectin peroxidase it was 1.5 μg/ml.

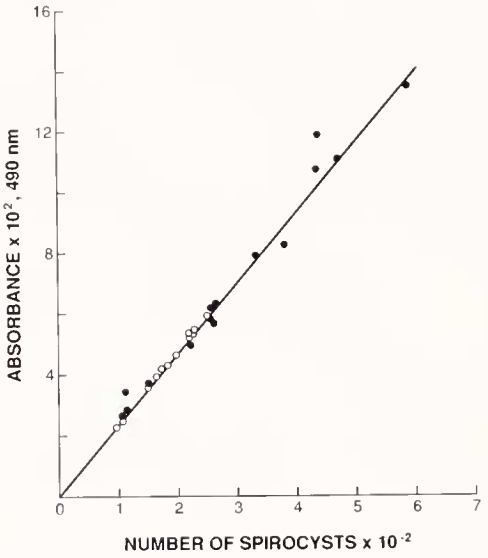

Figure 1. Standard curve for ELLSA determination of discharged spirocysts. The number of discharged spirocysts counted on test probes is plotted against absorbance at 490 nm. Solid circles represent mean values obtained from tentacles chemosensitized by N-acetylneuraminic acid and open circles from histamine-sensitized animals. Each point is the mean of separate absorbancy readings (n = 24) and direct countings (n = 11) (R = 0.99).

Standard curve

A standard curve was constructed by plotting visually counted spirocysts per probe as a function of absorbance at 490 nm. Visual counts of spirocysts discharged onto probes from animals that were chemosensitized by various concentrations of either NANA or histamine were performed under phase contrast optics. Direct counts and absorbancy readings were obtained using replicate probes from the same animals. For absorbancy readings, a total of four separate experiments were performed and averaged. Each experiment consisted of six replicate probes. A linear and direct relationship existed between the absorbance and the visually counted discharged spirocysts (Fig. 1).

Adhesive force measurements

Dose-response curves, expressing the mean adhesive force for all tested chemosensitizers, are biphasic. The curves for glycine, histamine, proline, and NANA exhibit a sigmoidal region of sensitization at low concentrations of sensitizer, a maximum response or effect (E_{max}) at higher concentrations (EC_{100}), and a region of apparent desensitization occurring at still higher concentra-

Table I

Dose-response parameters of agonist-sensitized cnida discharge and adhesive force measurements from Aiptasia pallida *tentacles*

	Spirocysts			Mastigophores			Adhesive force		
Agonists	E_{max} (no.)	EC_{100}	$K_{0.5}$ (M)	E_{max} (no.)	EC_{100}	$K_{0.5}$ (M)	E_{max} (mgf)	EC_{100}	$K_{0.5}$ (M)
Glycine	107 ± 15	5×10^{-11}	$5.4 \times 10^{-12} \pm 0$	160 ± 24	10^{-6}	$1.2 \times 10^{-8} \pm 0.23$	8.7 ± 0.7	10^{-6}	$2.0 \times 10^{-8} \pm 0.2$
Histamine							12.0 ± 0.9	2.7×10^{-7}	$1.4 \times 10^{-8} \pm 0.1$
Peak 1	138 ± 18	2.7×10^{-9}	$9.7 \times 10^{-10} \pm 1.0$	110 ± 23	10^{-9}	$1.6 \times 10^{-10} \pm 0$			
Peak 2	159 ± 23	2.7×10^{-6}	$1.0 \times 10^{-7} \pm 0.3$	201 ± 40	10^{-7}	$1.9 \times 10^{-9} \pm 0.3$			
Proline							10.7 ± 1.0	10^{-6}	$3.6 \times 10^{-8} \pm 0.4$
Peak 1	128 ± 6	2.7×10^{-8}	$3.2 \times 10^{-9} \pm 0.2$	70 ± 15	10^{-8}	$5.4 \times 10^{-9} \pm 0.3$			
Peak 2	93 ± 7	2.7×10^{-6}	$1.7 \times 10^{-7} \pm 0.3$	86 ± 4	10^{-6}	$5.0 \times 10^{-7} \pm 0$			
NANA				157 ± 9	10^{-5}	$8.1 \times 10^{-9} \pm 0.5$	14.0 ± 1.0	1.8×10^{-5}	$3.6 \times 10^{-7} \pm 0.5$
Peak 1	233 ± 15	10^{-8}	$5.0 \times 10^{-9} \pm 0$						
Peak 2	396 ± 9	10^{-7}	$8.0 \times 10^{-8} \pm 0.4$						
Peak 3	172 ± 46	10^{-5}	$3.2 \times 10^{-6} \pm 1.0$						

E_{max} (no.) represents the maximal number of cnidae discharged onto single test probes at optimal sensitization. EC_{100} is the molar concentration of agonist producing a maximal effect. This value was obtained by visual inspection of dose-response curves. $K_{0.5}$ (M) represents the molar concentration of agonist producing the half-maximal effects. E_{max} (mgf) represents the maximal cnida-mediated adhesive force at optimal sensitization. Both E_{max} and $K_{0.5}$ values are determined from least-square double reciprocal plot of the sensitized region of the dose-response curve. Values represent the response to agonists alone (*i.e.*, controls subtracted) and are means ± standard error of the mean.

tions (Figs. 2A, 3A, 4A, and 5A, respectively). The dose-response curves differ with regard to the specific dose-response parameters (Table I): E_{max}, the maximum effect; $K_{0.5}$, the dose at which a half-maximum effect occurs; and EC_{100}, the dose at which the maximum effect occurs.

Dose-responses of mastigophore and spirocyst discharge

Glycine. The dose-response curves representing the discharge of mastigophores (Fig. 2B) and spirocysts (Fig. 2C) to glycine are biphasic. The dose-response of the discharge of spirocysts to glycine consists of a single modal dose-response similar to that obtained from adhesive force measurements and from the discharge of mastigophores (Fig. 2A, B). However, there are significant differences in the dose-responses of these two types of cnidae. The response of spirocysts sensitized by glycine is shifted significantly to the left of the glycine-sensitized mastigophore response, indicating that responding spirocytes are approximately 10,000 times more sensitive to glycine than are the responding mastigophore-bearing cnidocytes (Table I).

Before chemosensitization, the mean number of discharged spirocysts on control probes was 116; after sensitization, the number rose to 214. This is equivalent to an average increase of 86%. Because insignificant spirocyst discharge occurs at higher concentrations, and because the dose-response for adhesive force and for discharged mastigophores coincide, it appears that the discharged mastigophores are the major contributors to glycine-induced adhesive force.

Histamine. The dose-responses of discharging spirocysts and mastigophores sensitized by histamine are bimodal, each displaying two biphasic peaks (Fig. 3) that are complementary and non-overlapping. The two peaks of discharging mastigophores each appear to be about ten times more sensitive to histamine than the corresponding two peaks of discharging spirocysts.

Proline. The dose-response curves of cnida discharge to proline (Fig. 4) are similar to those obtained for histamine. Both the mastigophore and spirocyst response profiles are bimodal, but unlike histamine, they are complementary and coincidental, rather than non-overlapping. The discharge of spirocysts is less sensitive to proline than to histamine (Table I).

N-acetylneuraminic acid (NANA). The pattern of discharge elicited by NANA for spirocysts is trimodal, but for mastigophores it is modal. This is in contrast to the responses elicited by the tested "amino" agonists in which agonist-induced patterns were similar for both spirocysts and mastigophores. Each of the three biphasic spirocyst responses is fairly narrow (Fig. 5C), in comparison to the mastigophore response (Fig. 5B), which spans a range of NANA concentrations of five to six orders of magnitude.

Effect of target hardness on retention of cnidae

To determine whether the hardness of the target contributes to the number of cnidae retained on target probes, we varied the concentrations of the gelatin used (5–50%; w/v) to coat target probes. We sensitized all anemones at 10^{-5} M NANA to assure that the number of discharging cnidae remained constant. Thus, the number of discharged cnidae retained on probes mea-

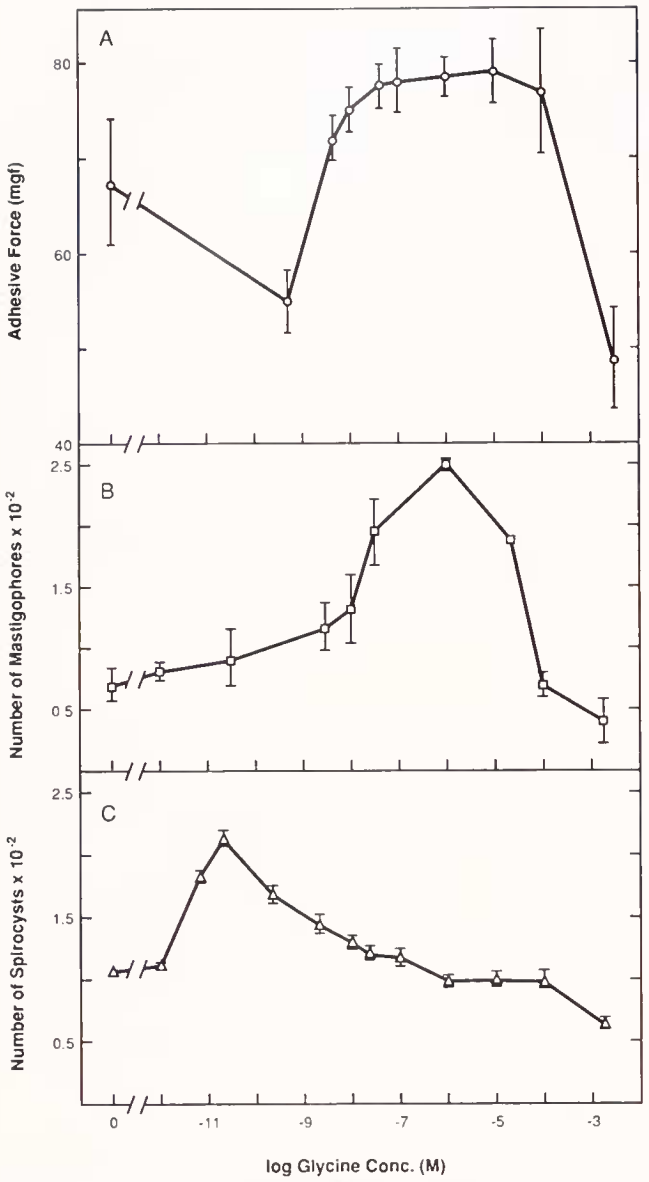

Figure 2. Dose-responses of glycine on discharge of cnidae. A. Effect of glycine on cnida-mediated adhesive force. Values express the mean of four separate experiments. Each experiment consists of eight replicate probes for each concentration; each probe and each tentacle is used only once (n = 32). B. Effect of glycine on the number of discharged mastigophores (n = 8). C. Effect of glycine on the number of discharged spirocysts (n = 24). The number of spirocysts was determined by the ELLSA assay. Vertical bars represent the standard error of the mean at 95% confidence limit.

sured the adhesion of the discharging cnidae to target surfaces of differing degrees of hardness.

We find that the retention of discharged mastigophores and spirocysts onto test probes of differing degrees of hardness is minimal at soft gelatin coatings of 5% (Fig. 6A, B). The adhesion curves with respect to gelatin concentration for retained mastigophores (Fig. 6A) and for adhesive force measurements (Fig. 6C) are biphasic, showing maxima at 40% and steep declines at 50%. The adhesion curve for spirocysts (Fig. 6B), on the other hand, is sigmoidal, reaching a maximum at 30% and then plateauing at harder coatings of gelatin.

At concentrations of gelatin below 20% the numbers of retained mastigophores predominated by as much as 2.5-fold (Fig. 6A, D). Approximately equal numbers of mastigophores and spirocysts were retained on probes

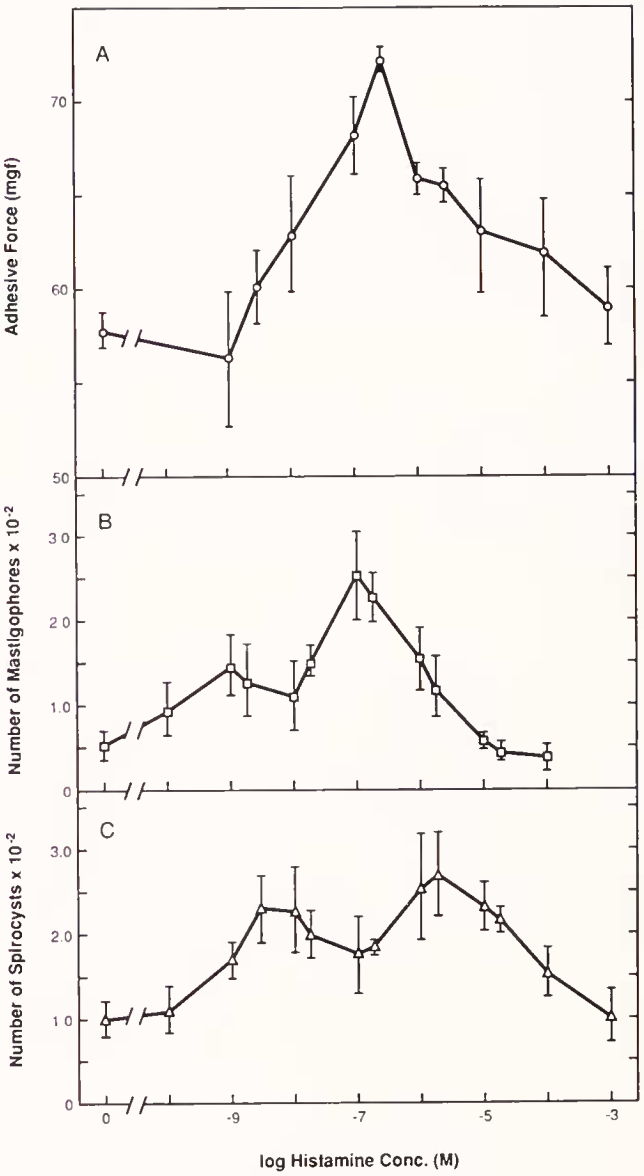

Figure 3. Dose-responses of histamine on discharge of cnidae. A. Effect of histamine on cnida-mediated adhesive force. Values express the mean of four experiments. Each experiment consists of eight replicate probes for each concentration; each probe and each tentacle is used only once (n = 32). B. Effect of histamine on the number of discharged mastigophores (n = 8). C. Effect of histamine on the number of discharged spirocysts (n = 24). The number of spirocysts was determined by the ELLSA assay on Figure 2C. Vertical bars represent the standard error of the mean at 95% confidence limit.

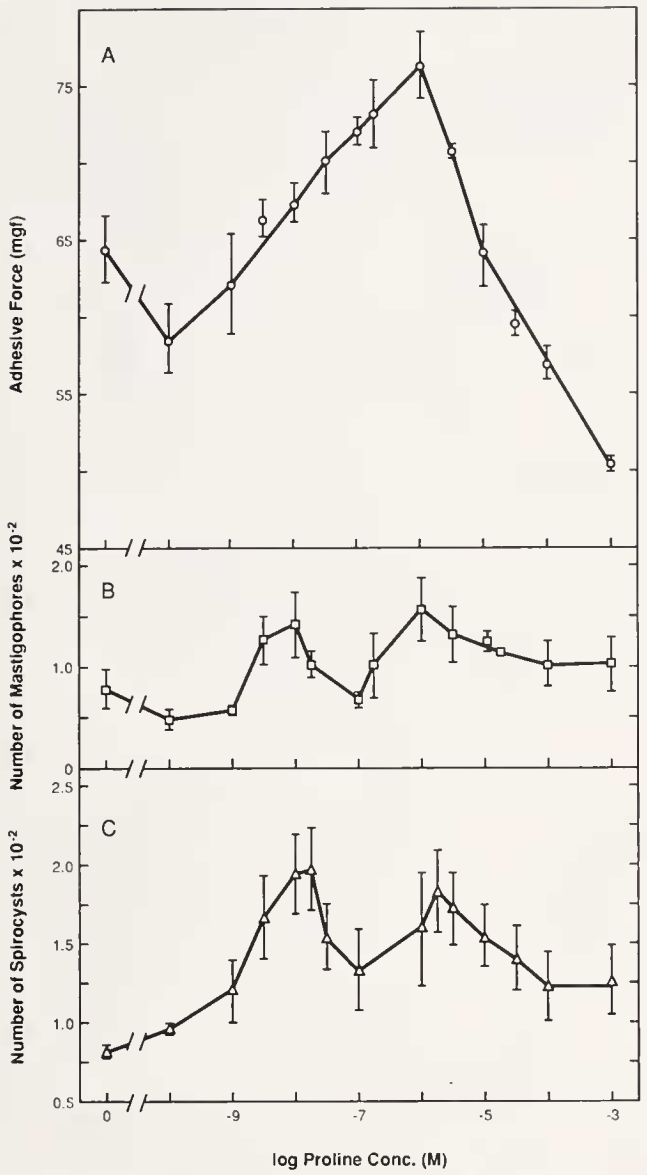

Figure 4. Dose-responses of proline on discharge of cnidae. A. Effect of proline on cnida-mediated adhesive force. Values express the mean of four experiments. Each experiment consists of eight replicate probes for each concentration; each probe and each tentacle is used only once (n = 32). B. Effect of proline on the number of discharged mastigophores (n = 7). C. Effect of proline on the number of discharged spirocysts (n = 24). The number of spirocysts was determined as in preceding figures. Vertical bars represent the standard error of the mean at 95% confidence limit.

coated with 20, 30 and 40% gelatin (Fig. 6D). However, the spirocysts predominated by about 3-fold at 50% gelatin (Fig. 6B, D).

Discussion

In the feeding tentacles of the sea anemone *Aiptasia pallida*, as in all acontiate anemones, three types of cnidae occur: the spirocysts, the microbasic p-mastigophores, and the basitrichous isorhizas (Hand, 1955). Recently, using cnida-mediated measurements of adhesive force in *A. pallida*, three different classes of chemoreceptors were identified that sensitize cnidocytes to discharge their cnidae in response to triggering mechanical stimuli (Thorington and Hessinger, 1988a, b). Although the discharge of the microbasic p-mastigophores is under the

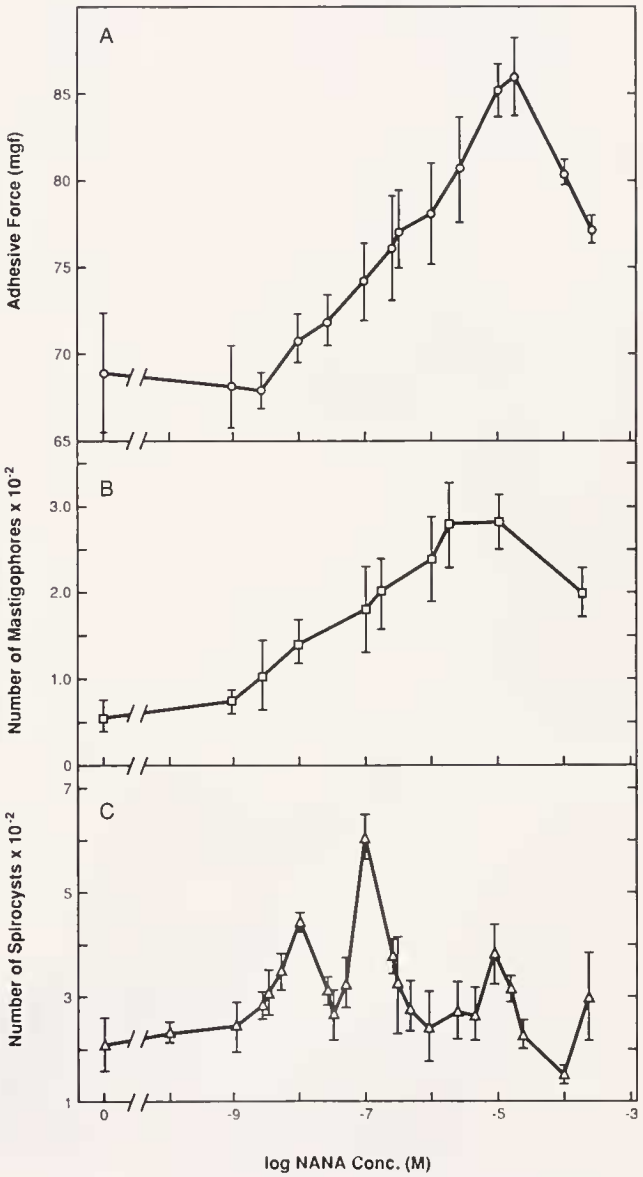

Figure 5. Dose-responses of N-acetylneuraminic acid (NANA) on discharge of cnidae. A. Effect of NANA on cnida-mediated adhesive force. Values express the mean of four experiments. Each experiment consists of eight replicate probes for each concentration; each probe and each tentacle is used only once (n = 32). B. Effect of NANA on the number of discharged mastigophores (n = 11). C. Effect of NANA on the number of discharged spirocysts (n = 24). The number of spirocysts was determined as in preceding figures. Vertical bars represent the standard error of the mean at 95% confidence limit.

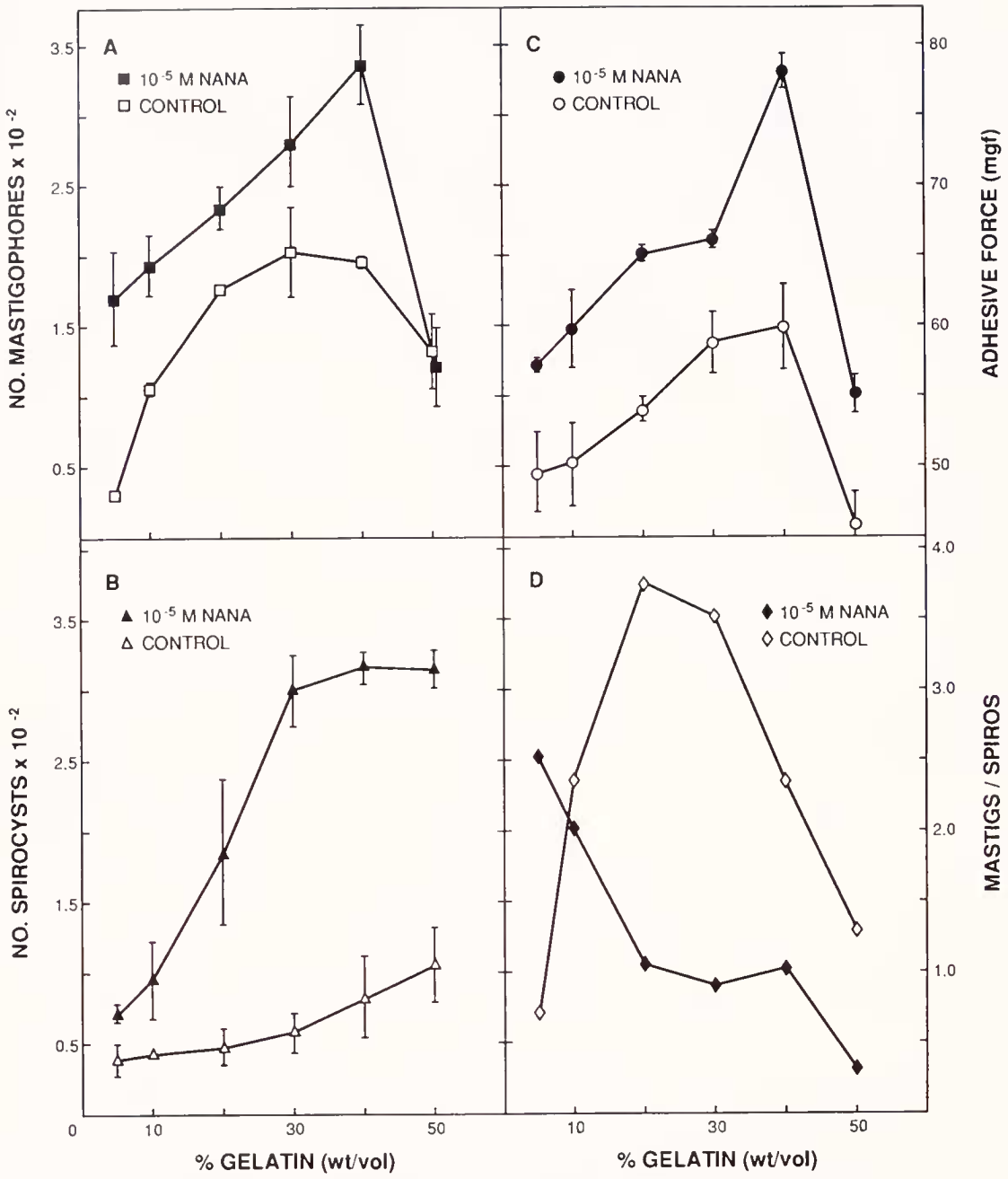

Figure 6. Dose-responses of retained discharged cnidae and of measured adhesive force using targets coated with varying concentrations of gelatin. A. Effect of target hardness on the number of mastigophores retained onto probes (n = 5). B. Effect of target hardness on the number of spirocysts retained onto probes (n = 5). C. Effect of target hardness on the measured adhesive force (n = 5). D. Ratio of retained mastigophores to retained spirocysts. All experiments were carried out either in 10^{-5} *M* N-acetylneuraminic acid or in seawater (controls). Data points are the mean ± standard error of the mean.

influence of at least two classes of sensitizing agonists, namely glycine and N-acetylated sugars (Geibel *et al.*, 1988), it is unknown whether such chemosensitizers, along with a third class of sensitizers, typified by heterocyclic amino compounds, also elicit similar responses from spirocytes.

Spirocysts have been described ultrastructurally (Mariscal and McLean, 1976; Mariscal *et al.*, 1976, 1977), but few experimental studies have been performed on spirocytes. The qualitative effects of remote mechanical stimuli (Conklin and Mariscal, 1976) and of food extracts (Williams, 1968) on the discharge of spiro-

cysts have been reported. Until now, the local, chemical control of spirocyst discharge and the purported primary role of spirocysts in retaining captured prey has not been quantitatively or experimentally verified. This lack of information is due in large part to the difficulty of detecting discharged spirocysts because they possess a highly transparent and non-refractile capsule. The counting of discharged spirocysts by optical methods is further complicated by the fact that the everted tubules entangle extensively. While the visibility of the capsules of discharged spirocysts is enhanced with phase contrast optics, the counting of these cnida is, nonetheless, tedious and time-consuming.

A rapid and sensitive assay of discharged spirocysts

To circumvent these problems, we developed a sensitive indirect, solid-state, enzyme-linked lectin sorbant assay (ELLSA) to detect discharged spirocysts. The assay is highly reproducible and is significantly faster than visually counting discharged spirocysts using phase contrast optics. The potential applications of this procedure include enumerating discharged spirocysts on experimental targets as well as detecting and characterizing the adhesive substance of spirocysts. In the present report we use this assay to study the effects on spirocyst discharge of two classes of substances known to sensitize the discharge of mastigophores (Geibel *et al.*, 1988), in addition to a third class of sensitizer known to sensitize cnida-mediated adhesive force (Thorington and Hessinger, 1988b).

Sensitization of spirocytes to discharge spirocysts

We have found that the three known classes of sensitizers as typified by glycine, NANA, and the heterocyclic amino compounds, histamine and proline, all sensitize spirocyst- and mastigophore-bearing CSCCs, albeit in very different and specific ways. In spite of the variability in sensitivity, magnitude, and pattern of spirocyte responsiveness induced by these agonists, each of the dose-response profiles consists of one or more biphasic peaks. Each biphasic peak reveals a region of sensitization reaching a maximal effect (E_{max}), followed by a region of desensitization at higher concentrations. The dose-response parameters (Table 1) indicate that the discharge of spirocysts is most sensitive to glycine, followed by histamine, proline, and then NANA, while the discharge of mastigophores is most sensitive to histamine, followed by proline, NANA, and glycine. The differences in the sensitivity of spirocytes and nematocytes to glycine were the most pronounced.

In addition to differences in sensitivity to agonists, the dose-response patterns also exhibited differences. In contrast to the modal (*i.e.* biphasic) dose-responses exhibited by measurements of adhesive force (Thorington and Hessinger, 1988a, b; Geibel *et al.*, 1988; Figs. 2A, 3A, 4A, 5A), we observe that dose-responses of the discharge of spirocysts to glycine is modal, while the dose-responses to proline and histamine are both bimodal, and the response to NANA is trimodal. These contrast to the dose-responses of discharging mastigophores, which for glycine and NANA are modal, while for proline and histamine are bimodal. Although the dose-responses of mastigophore and spirocyst discharge are not coincidental for any of the tested agonists, except possibly proline, the fact that all of the adhesive force dose-response curves are coincidental with those of the mastigophores implies that discharging mastigophores contribute significantly more to adhesive force than do discharging spirocysts.

Are all of the receptors effecting multimodal responses (*i.e.*, NANA) associated directly with the cnidocytes or possibly located on remote sites where they exert indirect control over cnidocyte responsiveness, such as via the nervous system or by initiating changes in behavior that affect the availability of cnidae to discharge? By using mucin-labelled colloidal gold, we find that 99.4% of the labelled gold binds to supporting cells adjacent to spirocytes and nematocytes (Watson and Hessinger, 1988), while no label binds to tentacle sensory cells. We conclude that the receptors to the multimodal agonist, NANA, are entirely located on supporting cells of CSCCs and not on remote sensory sites.

A salient feature of modal dose-responses is that the response is "turned off" at concentrations of agonist exceeding those needed to evoke a maximum response. Where multimodal responses are exhibited, high concentrations of agonist turn off the response of CSCCs having dose-response maxima below that concentration. The existence of bimodal and, particularly, trimodal dose-responses provides for discharge of cnidae over a wide range of agonist concentrations while ensuring that only a portion of the available CSCCs are sensitized at any one time and dose. Thus, the total number of discharging cnidae never reaches the total number present. This effectively conserves cnidae by preventing both excessive discharge against living prey and nonproductive discharge against killed prey.

Multiple populations of cnidocyte/supporting cell complexes (CSCCs)

The display of bimodal and trimodal dose-responses implies the existence of multiple populations of spirocytes distinguished by different sensitivities (*i.e.*, $K_{0.5}$ values) to a given agonist. That multiple populations of CSCCs exist is indicated by the fact that there are CSCCs, termed type C CSCCs, that discharge their cnidae in response to tactile stimuli in the absence of added agonist (Figs. 2, 3, 4, 5), in addition to CSCCs, termed type B

CSCCs, that require chemosensitization by agonists before they can be triggered to discharge by static (*i.e.*, non-vibrating) targets. Furthermore, mastigophore-bearing CSCCs triggered by targets vibrating at specific frequencies (Watson and Hessinger, 1989) are termed type A CSCCs. Although we do not yet know if vibration-sensitive, spirocyst-bearing type A CSCCs exist, there obviously exist different populations of spirocyst- and nematocyst-containing CSCCs distinguished by differences in their sensitivities and specificities to agonists and by the ways they are triggered by mechanical stimuli to discharge their cnidae.

Roles of discharged mastigophores and spirocysts in the capture of prey

In the light of our current findings, the commonly accepted roles of the spirocysts and the mastigophores in the capture and adherence of prey must be re-evaluated and modified. We consider these matters from the perspectives of two questions addressed by this report: (i) which of the two kinds of discharged cnida contribute most to cnida-mediated adhesive force; and (ii) which physical types of target retain the two kinds of cnida.

To adequately address the first question, we must recognize that the cnida-mediated components of adhesive force measurements reflect both the number and the kinds of cnidae discharging onto targets. A quantitative analysis of the contributions and magnitudes of these individual factors to adhesive force is beyond the scope of the present discussion, but we can make preliminary qualitative assessments based upon the findings presented here. We have seen for several agonists that the dose-responses for adhesive force measurements and for the number of discharged mastigophores coincide and more closely resemble each other than do the dose-responses for discharging spirocysts (Figs. 2–5). This is also seen by comparing EC_{100} values for the discharge of cnidae with those for adhesive force (Table I). To assess the second question, we performed measurements of adhesive force and cnidae discharge in which the hardness of the gelatin-coating on the target probes was varied. With gelatin coatings below 20%, the number of discharged mastigophores retained on the target probes predominated over spirocysts (Fig. 6A, D), presumably because proportionally fewer discharging spirocysts can adhere to the "softer" targets. Equal and maximal numbers of mastigophores and spirocysts are retained on probes coated with 20, 30, and 40% gelatin (Fig. 6D). Above 40% gelatin, however, the spirocysts predominate (Fig. 6B, D), presumably because mastigophores are incapable of penetrating these "harder" targets. Thus, when the targets are too soft for the discharging spirocysts to adhere, the penetrant nematocysts predominate as the kind of cnida retained on the target and, collectively, they are the primary contributors to measured adhesive force. On the other hand, when targets are too hard for the discharging mastigophores to penetrate, then the spirocysts predominate as the retained cnida and, collectively, they provide the major contribution to adhesive force. Thus, the correlation between measured adhesive force and the number of discharging mastigophores on both dose-responsive curves and on adhesion curves suggests that discharged mastigophores contribute significantly more to adhesive force than do discharged spirocysts under conditions in which the target is penetrable to discharging mastigophores.

Conclusions

In this paper, we show that the discharge of spirocysts is chemosensitized by the same agonists that sensitize the discharge of mastigophores. That is not to say that there may not also exist agonists that sensitize only the discharge of mastigophores or of spirocysts. However, the dose-responses of these two kinds of cnidae differ both qualitatively and quantitatively.

The dose-responses of discharging mastigophores are either modal (*e.g.*, to glycine and NANA) or bimodal (*e.g.*, to proline and histamine), and are coincidental to dose-responses obtained from measuring adhesive force under the same conditions. We have presented strong evidence that the similarity between the dose-response curves of adhesive force measurements and the discharge of mastigophores is due to the discharged mastigophores contributing significantly more to cnida-mediated adherence onto 30% gelatin-coated targets than the discharged spirocysts.

It seems appropriate, therefore, to modify the purported roles of penetrant microbasic p-mastigophores and adhesive spirocysts in the capture of prey. Spirocysts have been generally regarded as the primary means by which adhesion of prey to the tentacle occurs (Williams, 1968; Doumenc, 1971; McFarlane and Shelton, 1975; Mariscal, 1984). Mastigophores have been regarded as primarily penetrating and envenomating prey while, by implication, not contributing significantly to prey adhesion unless the tubules wrap around bristles or projections on prey (Mariscal, 1984). Our findings, however, indicate that mastigophores play a significant, and sometimes primary, role in the adhesion of prey, depending most likely upon the hardness of the prey surface. Indeed, it appears that mastigophores and spirocysts may be complimentary in their relative contributions to prey adhesion so that the contribution of mastigophores to adhesive force predominates with soft-surfaced targets, which they can penetrate. The contribution of spirocysts to adhesive force predominates when the target surface is hard enough for the spirocysts to adhere and too hard for the mastigophores to penetrate. Thus, in addition to

penetrating and immobilizing prey, discharging mastigophores contribute significantly, even predominantly, to the adhesion of prey, provided they are able to penetrate the surface of the prey.

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Literature Cited

Bigger, C. H. 1982. The cellular basis of the aggressive acrorhagial response of sea anemones. *J. Morphol.* **173:** 259–278.

Conklin, E. J., and R. N. Mariscal. 1976. Increase in nematocyst and spirocyst discharge in a sea anemone in response to mechanical stimulation. Pp. 549–558 in *Coelentrate Ecology and Behaviour,* G. O. Mackie, ed. Plenum Press, New York.

Doumenc, D. 1971. Aspects morphologiques de la dévagination du spirocyste chez *Actinia equina. L. J. Microsc.* **12:** 263–270.

Ewer, R. F. 1947. On the functions and mode of action of the nematocysts of hydra. *Proc. Zool. Soc. Lond.* **117:** 365–376.

Francis, L. 1973. Intraspecific aggression and its effect on the distribution of *Anthopleura elegantissima* and some related sea anemones. *Biol. Bull.* **144:** 73–92.

Geibel, G., G. Thorington, R. Y. Lim, and D. A. Hessinger. 1988. Control of cnida discharge: II. Microbasic p-mastigophore nematocysts are regulated by two classes of chemoreceptors. *Biol. Bull.* **175:** 132–136.

Hand, C. 1955. The sea anemones of central California. Part III. The acontiarian anemones. *Wasmann J. Biol.* **13:** 189–251.

Mariscal, R. N. 1972. The nature of adhesion to shells of the symbiotic sea anemone *Calliactis tricolor.* (Leseur). *J. Exp. Mar. Bio. Ecol.* **8:** 217–224.

Mariscal, R. N. 1974. Nematocysts. Pp. 129–178 in *Coelenterate Biology: Reviews and New Perspectives,* L. Muscatine and H. M. Lenhoff, eds. Academic Press, New York.

Mariscal, R. N. 1984. Cnidaria: Cnidae. Pp. 57–68 in *Biology of the Integument,* Vol. I. Invertebrates, J. Bereiter-Hahn, A. G. Maltolsy, and K. S. Richards, eds. Springer-Verlag, Berlin.

Mariscal, R. N., and R. B. McLean. 1976. The form and function of cnidarian spirocysts. II. Ultrastructure of the tip and wall and mechanism of discharge. *Cell Tissue Res.* **169:** 313–321.

Mariscal, R. N., C. H. Bigger, and R. B. McLean. 1976. The form and function of cnidarian spirocysts. I. Ultrastructure of the capsule exterior and relationship to the tentacles sensory surface. *Cell Tissue Res.* **168:** 465–474.

Mariscal, R. N., R. B. McLean, and C. Hand. 1977. The form and function of cnidarian spirocysts. III. Ultrastructure of the thread and the function of spirocysts. *Cell Tissue Res.* **178:** 427–433.

Mariscal, R. N., E. J. Conklin, and C. H. Bigger. 1978. The putative sensory receptors associated with the cnidae of cnidarians. *Scanning Electron Microsc.* **2:** 959–966.

McFarlane, I. D., and G. A. B. Shelton. 1975. The nature of the adhesion of tentacles to shells during shell-climbing in the sea anemone *Calliactis parasitica* (Couch). *J. Exp. Mar. Biol. Ecol.* **17:** 177–186.

Picken, L. E. R., and R. J. Skaer. 1966. A review of researches on nematocysts. In *The Cnidarians and Their Evolution,* W. J. Rees, ed. *Symp. Zool. Soc. Lond.* **16:** 15–50.

Purcell, J. E. 1977. Aggressive function and induced development of catch tentacles in the sea anemone *Metridium senile* (Coelenterata, Actiniaria). *Biol. Bull.* **153:** 355–368.

Skaer, R. J., and L. E. R. Picken. 1965. The structure of the nematocyst thread and the geometry of discharge in *Corynactis viridis.* (Allman). *Phil. Trans. Roy. Soc. Lond.* **250:** 131–164.

Stephenson, T. A. 1929. On the nematocysts of sea anemones. *J. Mar. Biol. Assoc. U.K.* **16:** 173–200.

Thorington, G. U., and D. A. Hessinger. 1988a. Control of cnida discharge: I. Evidence for two classes of chemoreceptors. *Biol. Bull.* **174:** 163–171.

Thorington, G., and D. A. Hessinger. 1988b. Control of discharge: factors affecting discharge of cnidae. Pp. 233–253 in *Biology of Nematocysts,* D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego.

Watson, G., and R. Mariscal. 1983. The development of a sea anemone tentacle specialized for aggression: morphogenesis and regression of the catch tentacle of *Haliplanella luciae* (Cnidaria, Anthozoa). *Biol. Bull.* **164:** 507–517.

Watson, G., and D. A. Hessinger. 1988. Localization of a purported chemoreceptor involved in triggering cnida discharge in sea anemones. Pp. 255–272 in *Biology of Nematocysts,* D. A. Hessinger and H. M. Lenhoff, eds. Academic Press, San Diego.

Watson, G., and D. A. Hessinger. 1989. Cnidocyte mechanoreceptors are tuned to the movements of swimming prey by chemoreceptors. *Science* **243:** 1589–1591.

Weill, R. 1934. Contribution a l'étude des cnidaires et de leurs nématocysts. I. Réchérches sur les nématocystes. *Trav. Sta. Zool. Wimereux* **10:** 1–347.

Williams, R. B. 1968. Control of the discharge of cnidae in *Diadumene luciae* (Verill). *Nature* **219:** 959.